

34. The method of claim 33, wherein said endonuclease recognition site is selected from the group consisting of Class I I-endonuclease sites, Class II I-endonuclease sites, Class III I-endonuclease sites, Class IV I-endonuclease sites, and Class V I-endonuclease sites.

35. The method of claim 34, wherein said endonuclease recognition site is a Class I I-endonuclease site.

36. The method of claim 35, wherein said endonuclease recognition site is selected from the group consisting of I-SceI, I-SceIV, I-CsmI, and I-PanI sites.

37. The method of claim 36, wherein said endonuclease recognition site is an I-SceI site. --

REMARKS

Entry and consideration of this amendment is respectfully requested.

Claims 1-26 have been canceled. New claims 27-37 find support throughout the specification, for example on pages 20-21 and Fig.6. Accordingly, no new matter is entered by amendment.

Applicants submit herewith a Sequence Listing and have amended the specification to conform with the requirements of 37 C.F.R. §§ 1.821-1.825.

Applicants request the use of the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, now U.S. Patent No. 5,962,327, issued October 5, 1999.

08/417-226

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I hereby state that the contents of the paper copy of the Sequence Listing in this application and the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Version with Markings to Show Changes MadeIn the Specification:

The paragraph beginning on line 4 of page 5 has been amended as follows:

Accordingly, this invention aids in fulfilling these needs in the art. Specifically, this invention relates to an isolated DNA encoding the enzyme I-SceI. The DNA has the following nucleotide sequence:

	ATG	CAT	ATG	AAA	AAC	ATC	AAA	AAA	AAC	CAG	GTA	ATG	2670
	M	H	M	K	N	I	K	K	N	Q	V	M	12
2671	AAC	CTC	GGT	CCG	AAC	TCT	AAA	CTG	CTG	AAA	GAA	TAC	AAA
13	N	L	G	P	N	S	K	L	L	K	E	Y	K
													2730
													32
2731	ATC	GAA	CAG	TTC	GAA	GCA	GGT	ATC	GGT	CTG	ATC	CTG	GGT
33	I	E	Q	F	E	A	G	I	G	L	I	L	G
													2790
													52
2791	GAT	GAA	GGT	AAA	ACC	TAC	TGT	ATG	CAG	TTC	GAG	TGG	AAA
53	D	E	G	K	T	Y	C	M	Q	F	E	W	K
													2850
													72
2851	GTA	TGT	CTG	CTG	TAC	GAT	CAG	TGG	GTA	CTG	TCC	CCG	CCG
73	V	C	L	L	Y	D	Q	W	V	L	S	P	P
													2910
													92
2911	CAC	CTG	GGT	AAC	CTG	GTA	ATC	ACC	TGG	GGC	GCC	CAG	ACT
93	H	L	G	N	L	V	I	T	W	G	A	Q	T
													2770
													112
2971	AAA	CTG	GCT	AAC	CTG	TTC	ATC	GTT	AAC	AAC	AAA	AAA	ACC
113	K	L	A	N	L	F	I	V	N	N	K	K	T
													3030
													132
3031	AAC	TAC	CTG	ACC	CCG	ATG	TCT	CTG	GCA	TAC	TGG	TTC	ATG
133	N	Y	L	T	P	M	S	L	A	Y	W	F	M
													3090
													152
3091	TAC	AAC	AAA	AAC	TCT	ACC	AAC	AAA	TCG	ATC	GTA	CTG	AAC
153	Y	N	K	N	S	T	N	K	S	I	V	L	N
													3150
													172
3151	GAA	GTA	GAA	TAC	CTG	GTT	AAG	GGT	CTG	CGT	AAC	AAA	TTC
173	E	V	E	Y	L	V	K	G	L	R	N	K	F
													3210
													192
3211	ATC	AAC	AAA	AAC	AAA	CCG	ATC	ATC	TAC	ATC	GAT	TCT	ATG
193	I	N	K	N	K	P	I	I	Y	I	D	S	M
													3270
													212
3271	CTG	ATC	AAA	CCG	TAC	CTG	ATC	CCG	CAG	ATG	ATG	TAC	AAA
213	L	I	K	P	Y	L	I	P	Q	M	M	Y	K
													3330
													232
3331	GAA	ACT	TTC	CTG	AAA	TAA	(SEQ ID NO:1)						
233	E	T	F	L	K	*	(SEQ ID NO:2).						

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On page 7 beginning at line 2, paragraphs 1-11 have been amended as follows:

This invention will be more fully described with reference to the drawings in which:

Fig. 1 depicts the universal code equivalent of the mitochondrial I-SceI gene (SEQ ID NO:1).

Fig. 2 depicts the nucleotide sequence of the invention encoding the enzyme I-SceI and the amino acid sequence of the natural I-SceI enzyme (SEQ ID NOS: 5 and 2).

Fig. 3 depicts the I-SceI recognition sequence and indicates the possible base mutations in the recognition site and the effect of such mutations on stringency of recognition (SEQ ID NOS: 6, 7, and 8).

Fig. 4 is the nucleotide sequence and deduced amino acid sequence of a region of plasmid pSCM525. The nucleotide sequence of the invention encoding the enzyme I-SceI is enclosed in the box (SEQ ID NOS: 9 through 16).

Fig. 5 depicts variations around the amino acid sequence of the enzyme I-SceI (SEQ ID NO: 2).

Fig. 6 shows Group I intron encoding endonucleases and related endonucleases (SEQ ID NOS: 17-44).

Fig. 7 depicts yeast expression vectors containing the synthetic gene for I-SceI.

Fig. 8 depicts the mammalian expression vector PRSV I-SceI.

Fig. 9 is a restriction map of the plasmid pAF100. (See also YEAST, 6:521-534, 1990, which is relied upon and incorporated by reference herein).

Figs. 10A and 10B show the nucleotide sequence and restriction sites of regions of the plasmid pAF100 (SEQ ID NOS: 45-50).

On page 12, the last paragraph has been amended as follows:

The enzyme I-SceI has a known recognition site. (ref. 14.) The recognition site of I-SceI is a non-symmetrical sequence that extends over 18 bp as determined by systematic mutational analysis. The sequence reads: (arrows indicate cuts)

↓

5'	TAGGGATAACAGGGTAAT	3'	(SEQ ID NO: 51)
3'	ATCCCTATTGTCCCATTA	5'	(SEQ ID NO: 52)

↑

T 0 3 F 1 0 : 0 9 T 9 E 5 5 0

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4B

On pages 41 to 42, the bridging paragraph has been amended as follows:

-e- The supernatant of this clone was used to infect other mouse cells (1009) by spreading 10^5 virus particles on 10^5 cells in DMEM medium with 10% fetal calf serum and 5 mg/ml of "[polybrain] polybrene (hexadimethrine bromide)". Medium was replaced 6 hours after infection by the same fresh medium.

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